REMARKS

This Amendment filed in response to the Non-Final Office Action dated February 6, 2007, is believed to be fully responsive to the objections and rejections raised therein. Accordingly, favorable reconsideration on the merits is respectfully requested.

In the present Amendment, claims 3 and 4 have been amended to improve its form.

No new matter has been added. Entry of the Amendment is respectfully requested. Upon entry of the Amendment, claims 1-4 will be all the claims pending in the application.

I. Response to Claim Rejections Under 35 U.S.C. §§ 102(a) and 103(a)

Claim 1 is rejected under 35 U.S.C. § 102(a) as being anticipated by Johnston et al. Blood, **2001 98** (11), p. 410 (hereinafter "Johnston et al."). Claims 1-4 are rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Johnston et al. in view of Rachmilewitz, British Journal of Haematology, 1995, 91, 263-268 (hereinafter "Rachmilewitz").

Beta-thalassemia and sickle-cell anemia (SCA) are hereditary diseases caused by mutations of the beta-globin gene and require life-long treatments. In the case of thalassemia major, the patients need to be regularly transfused. The most relevant therapeutic options and clinical trials for beta-thalassemia patients include: blood transfusion, bone marrow transplantation, gene therapy, and induction of fetal hemoglobin ("HbF").

The subject matter of Applicants' application is directed to induction of HbF. It is submitted that the subject matter of claim 1 is novel and therefore patentable over the art of record because rapamycin induces the expression of HbF in human erythroid cells from beta-thalassemia patients. Even if several HbF inducers are already known (including recombinant erythropoietin), it is common opinion that novel inducers are needed for at least two reasons: (a)

toxicity of some of the described inducers and (b) the fact that several patients are not-responders or become not-responders after long-term therapy. It should be pointed out that pharmacological therapy of beta-thalassaemia is expected to be crucial for several developing countries, unable to efficiently sustain the high-cost clinical management of beta-thalassemia patients requiring regular transfusion regimen, chelation therapy and advanced hospital facilities. Furthermore, it is well known that, in addition of "direct costs", blood transfusions require accurate monitoring of the blood safety, by using costly technologies, some of which are based on multiple PCR covering all the possible hematological infectious diseases. The HbF induction therapeutic approach overcomes these drawbacks of transfusion treatment.

As far as clinical trials, aimed at increasing HbF synthesis in beta-thalassemia and SCA patients, they have included administration of cell-cycle-specific agents, hematopoietic growth factors and short-chain fatty acids, all of which stimulate -globin synthesis by different mechanisms. Compounds such as 5-azacytidine, hydroxyurea (HU), and butyrate analogues have been the most frequently used. When all the clinical data are considered together, it appears that HbF inducers are clinically beneficial for patients affected by beta-thalassemia and SCA. However, this approach (in the case of HU) was not useful in several patients (about 50%).

The approach described by Johnston et al. (Blood, 98, 410) deals with gene therapy. They developed a gene therapy system based on inducible AAV vectors. The vectors carry a "therapeutic gene" (in the described case the erythropoietin gene, but the technology can be applied to many other genes) under the control of a transcription activation system. This is of interest in consideration of the fact that some proteins, if overproduced following gene therapy, might be potentially harmful. For instance, in the case of AAV vectors expressing the

coagulation Factor IX, no need for a programmed activation is required, since Factor IX has a good safety profile. On the contrary, EPO, if overproduced, might cause severe and even fatal complications. Therefore, the equipment of the vector/transgene system with a regulatory system is essential, in order to allow pharmacologically controlled expression. Several controlled systems have been described, including those controlled by an engineered transcription factor induced by tetracycline (Tet), mifepristone or ecdysone. Johnston et al. (Blood, 98, 410) developed a system in which rapamycin is used as a bivalent "dimerizer" drug with the aim to reversibly cross-link the DNA-binding and activation domains of a transcription factor expressed separately as fusion proteins. Addition of rapamycin dimerizes the fusion proteins activating transcription of the target gene (Figure 1).

As a proof of principle of the method, they demonstrated that rapamycin, combined with injection of AAV vectors expressing EPO under rapamycin control, improved anemia in a mouse model of beta-thalassemia. This was expected, since EPO is known to sustain erythropoiesis. Accordingly, the amelioration of anemia in the mouse model of beta-thalassemia following rapamycin treatment of AAV-EPO treated mice is dependent on the high-level of induction of erythropoietin, leading to a potentiation of erythropoiesis.

There is no teaching by Johnston et al. (Blood, 98, 410) that rapamycin would be beneficial to thalassemic patients as HbF inducer. Rapamycin is presumably needed only in AAV-treated patients in order to induce the expression of the transgene in a controlled fashion. Johnston et al., indeed, demonstrate beneficial effects in beta-thalassemia mice injected with the therapeutic AAV-vector containing the EPO gene under the control of rapamycin activated engineered chimeric transcription factors FRAP-p65 and ZFHD1-FKBP (see scheme of Figure 1). The transcription factors activated by rapamycin are not natural occurring

transcription factors, but are engineered chimeric molecules retaining a transactivation domain, a nuclear localizing signal and rapamycin-binding domains (FRAP and FKBP) allowing dimerization. Accordingly, the study by Johnston et al. does not anticipate an effect of rapamycin in inducing endogenous EPO, limiting the rapamycin effects to their gene-therapy system.

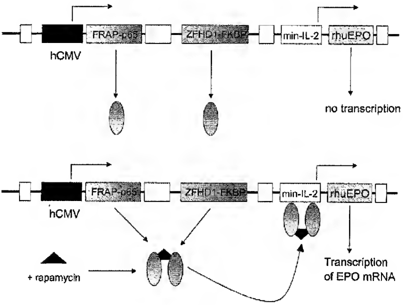

Figure 1. Scheme of the protocol developed by Johnston et al. (Blood, 98, 410), based of the rapamycin-mediated dimerization of artificially engineered transcription factors carrying FRAP and FKBP domains.

Unlike the approach described by Johnston et al. (Blood, 98, 410), the present invention demonstrates that rapamycin retains a novel and unpredictable effect, which is the induction of HbF in beta-thalassemic cells not treated with gene therapy vectors or other inducers. This activity of rapamycin occurs in the absence of transfection with AAV-based DNA, is unexpected, and allows including rapamycin within the list of HbF inducers. As reviewed by Rachmilewitz (British Journal of Haematology, 1995) other inducers are known (including EPO) that might exhibit synergism with rapamycin-mediated effects. In Applicants' application, the

novelty of the use of known inducers of HbF is the possible combination with rapamycin. Applicants emphasize that the activation of EPO in AAV-treated animals occurs only because they are treated with the therapeutic AAV vector containing also gene sequences encoding the engineered chimeric TFs that are activated by rapamycin through dimerization.

The finding that rapamycin induces HbF is novel and unexpected. Rapamycin (as Sirolimus or Rapamune) has been approved by the U.S. Food and Drug Administration for prevention of acute rejection in renal transplant recipients. There is no mention of the use of rapamycin to increase HbF in erythroid progenitors. In addition, rapamycin mediated effects are due to a well known mechanism of action. Rapamycin, indeed, has a specific molecular target (FKBP12, FK-506 binding protein). The rapamycin/FKBP12 complex interacts with mTOR (mammalian Target Of Rapamycin, known also as FRAP). mTOR has the important role of phosphorylation of p70 S6 kinase and 4E-BP1 to release active eIF-4E. FKBP-12 complexed with rapamycin inhibits mTOR and prevents these essential responses. No information was available, at the time of invention, on the possible effects of these pathways on HbF production.

For the above reasons, it is respectfully submitted that rapamycin induces HbF is beta-thalassemia patients is novel, and unexpected. Furthermore, it is submitted that rapamycin mediated induction is of great interest for the therapy of thalassemia and SCA, since its mechanism of action is expected to be different from the other known HbF inducers. Thus, Applicants respectfully request reconsideration of the rejection in view of the above arguments in response to the rejection. Further, Applicants request that the rejection of claim 1 be withdrawn.

Claims 2-4, depend from claim 1 and are patentable over Johnston et al. for at least all the above-mentioned reasons. Thus, Applicants respectfully request withdrawal of the rejections of claims 2-4.

II. <u>Response to Claim Rejection Under 35 U.S.C. § 112, Second Paragraph</u>

Claims 3 and 4 are rejected under 35 U.S.C. § 112, second paragraph for lack of antecedent basis. Claims 3 and 4 have been amended to obviate the rejection. Applicants request that the rejection be withdrawn in view of the amendment.

III. <u>Conclusion</u>

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

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